

# Infectious Agents and Cancer

Research article

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## Transcription profile of a human breast cancer cell line expressing MMTV-like sequences

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### Abstract

**Background:** It has been postulated that inflammation caused by certain viruses might result in cancer. Recently, it was shown that childhood lymphoblastic leukemia, breast and ovarian cancers express an interferon-related signature, providing support for this notion. We have previously shown that 38% of the sporadic breast cancers contain MMTV-like *env* gene sequences. To find out if the presence and expression of MMTV-like sequences correlated with an inflammatory phenotype, we have compared the expression profile of two sublines of MCF-7 cells, one containing the MMTV-like sequences (*env*<sup>+</sup>), the other one lacking them (*env*<sup>-</sup>).

**Results:** The results indicated that there were 47 differentially expressed genes between the two sublines. Among 27 upregulated genes in the *env*<sup>+</sup> cells there were 7 interferon-related genes, 5 TNF-connected genes and 2 TGF $\beta$ -related genes.

**Conclusion:** These results suggest that the *env*<sup>+</sup> cells were most likely responding to an infectious agent, and support the hypothesis that a viral infection may play a role in breast cancer pathogenesis.

### Background

We and others [1-4] have shown that 37 to 41% of sporadic breast cancer samples contain MMTV-like *env* gene sequences. The sequences are expressed as RNA [5] and as protein in breast cancers (Melana *et al.*, submitted). They are absent from the normal breasts of patients with *env* positive tumors [6] and are expressed as RNA exclusively in the cancer cells [7]. The whole proviral structure, designated human mammary tumor virus (HMTV), which has 95% homology to MMTV, can be detected in two tumors [8]. Although sequence variations are observed in the C-

terminal of human *sag* sequences, the cloned human *sag* sequences expressed in human B lymphocytes can activate human T-cells, as can the mouse *Sag*, indicating that it can be functional [9]. Moreover, viral particles with the morphological characteristics of betaretroviruses are observed in primary cultures of human breast cancer [10]. Taken together, these results suggest that an infectious agent is present in some human breast cancers.

Chronic inflammation has been implicated in tumor progression. New evidence suggests that the inflammation

caused by certain viruses results in cancer [Reviewed in [11]]. Recently, it was reported that childhood lymphoblastic leukemia, as well as breast and ovarian cancers express an interferon-related signature, but not found in other human cancers studied [12]. This finding provides molecular support for the role of inflammation or viral infection in cancer pathogenesis [12].

The established breast cancer cell line MCF-7 is widely used in research, and many subclones are available. Some of the original isolates produce retroviral-like particles [13]. Furthermore, May and Westerly [14] described the presence of an MMTV-like 6.6 Kb EcoR1 fragment in some of the MCF-7 cell lines, which was absent in other breast cancer lines and in normal tissue.

Continuous passage with subsequent chromosomal change [15] may have eliminated viral sequences from some of them. It has been reported that some sublines of MCF-7 show biological differences [16] and significant genetic variation in RNA expression [17,18].

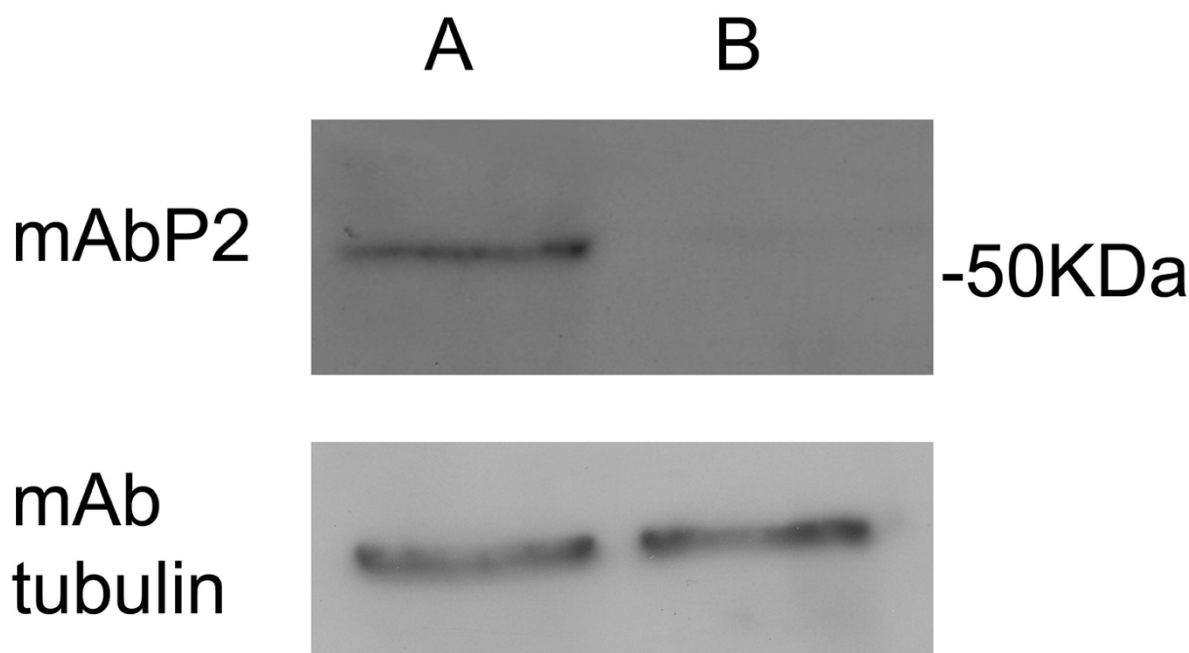
We have previously reported that a subline of MCF-7 containing *env* and LTR sequences [19,20] and that it expressed the *env* gene as RNA [7], while other sublines were negative for *env* gene [[21] and our own results]. To find out whether the presence of viral sequences is related

to an interferon-related signature, we have compared the expression profiles of two sublines of MCF-7 [22], one which contains the MMTV-like *env* gene sequence (*env*+) and one which lacks it (*env*-).

## Results

The presence of the Env protein was investigated in both sublines. In Fig. 1 the result of the immunoblotting experiment is shown. The HMTV *env*+ cell line expressed a protein of a MW of approximately 50 kD which reacted with mAbP2, a monoclonal antibody against a synthetic peptide derived from human *env* sequences (Melana *et al* submitted). It was absent in the HMTV *env*- cells. Tubulin was equally present in both extracts.

The results of the cDNA arrays are shown in Tables 1 and 2. Nineteen genes showed a > 2.5 fold difference in their adjusted intensity between HMTV *env*+ and *env*- cells, while another eight genes were only expressed in the HMTV *env*+ cells (Table 1). Twenty genes were downregulated (Table 2) in HMTV *env* + cells. Taken together, there were 47 differentially expressed genes. Among the 27 upregulated genes there were six interferon-inducible ones: IFI6, TRIM22, IFITM1, IFITM2+IFITM3, IFI27 and IP-30 and a receptor IFNGR2. In addition, there were five upregulated genes that have a connection with TNF or are involved in its signaling, like LTBR, TRAF3, MMP17,



**Figure 1**

Western blot of MCF-7 cells. Experimental conditions as described in Materials and Methods. A: MCF-7 (+) cells; B: MCF-7(-) cells.

PKN1 and MAPK13. The cytokine TGF $\beta$ , and its downstream effector early growth response protein 1 (EGR1), were also upregulated in *env+* cells. Twenty genes were down regulated in HMTV *env+* cells.

## Discussion

Comparison of the expression profiles of sublines derived from the same cell line provides an excellent model with minimal differences. Karyogenetic analysis revealed that the two sublines have similar complex chromosomal patterns (not shown). The comparison of expression profiles of MCF-7 *env+* and *env-* cells indicated preferential expression of interferon-related genes: 26% (7/27) of the up-regulated genes. These differences may indicate a trend. Einav *et al.* [12] have reported that 40% of clinical breast cancer samples display an interferon-associated signature; 17 out of 36 (47%) of the upregulated genes. Our results are consistent with, but cannot be directly compared with those of Einav's for several reasons: we used only one cell line for analysis, the participation of stroma and surrounding tissues has been eliminated from our study, and finally, we used a different set of arrays. Nevertheless, our results strongly indicate that HMTV *env+* MCF-7 cells express more interferon-related genes than the HMTV *env-* MCF-7 cells, suggesting that they may be responding to an infectious agent as proposed by Einav *et al.* [12]. The expres-

sion profile of HMTV *env+* cells suggests an increased potential for cell growth, a fact that may be related to their more malignant phenotype as has been described in breast cancer cells associated with HMTV [23,3,24]. It is remarkable that the alpha 7 and beta 4 integrins were significantly down regulated in *env+* cells, as has been reported in a set of finite life-span metastatic breast cancer cells which were also *env+* [25].

Whether the HMTV works as initiator and/or as promoter of malignant growth is uncertain. Molecular evidence that HMTV expression is responsible for the increase in interferon-related expression is being sought.

## Conclusion

The results clearly indicate that the transcriptional profile of the cells expressing HMTV sequences is enriched in genes involved in inflammation process. This finding is significant because it was obtained comparing cells derived from the same cell line that have similar genetic background and minimal expressing differences. This supports the hypothesis that a viral infection may play a role in breast cancer pathogenesis.

**Table 1: Up-regulated genes in HMTV *env+* cells**

Acc #	Gene symbol	Protein/gene	Ratio	Diff
<u>X02492</u>	IFI6	Interferon-inducible protein 6	12.44	4108
<u>J05633</u>	ITGB5	Integrin beta 5	9.46	3401
<u>X82200</u>	TRIM22	Tripartite motif-containing 22	5.76	1495
<u>L03840</u>	FGFR4	Fibroblast growth factor receptor 4	5.72	1742
<u>J04164</u>	IFITM1	Interferon induced transmembrane protein 1 (9-27)	5.38	6776
<u>M64595</u>		small G protein	4.74	1720
<u>X57351</u>	IFITM2 + IFITM3	interferon induced transmembrane prot 2 (1-8D) + 3 (1-8U)	4.25	1082
<u>X89576</u>	MMP17	matrix metalloproteinase 17	4.20	1647
<u>X52541</u>	EGR1	early growth response protein 1	3.39	1327
<u>L29220</u>	CLK3	CDC-like kinase 3	3.38	1521
<u>X66362</u>	PCTK3	PCTAIRE protein kinase 3	3.34	1368
<u>U33053</u>	PKN1	protein kinase N1	2.85	1075
<u>L04270</u>	LTBR	lymphotoxin beta receptor (TNFR superfamily, member3)	2.79	1406
<u>U09579</u>	CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	2.69	6193
<u>U14966</u>	RPL5	60S ribosomal protein L5	2.68	1641
<u>M29039</u>	JUNB	jun-B	2.65	1313
<u>M29971</u>	MGMT	6-O-methylguanine-DNA methyltransferase	2.63	1971
<u>M65199</u>	EDN2	endothelin 2	2.58	1535
<u>U57342</u>	MLF2	myeloid leukemia factor 2	2.52	2897
<u>X69398</u>	CD47	CD47 glycoprotein; integrin-associated protein	Up	4152
<u>U12255</u>	FCGRT	Fc fragment of IgG, receptor, transporter	Up	2960
<u>AF004709</u>	MAPK13	mitogen-activated protein kinase 13	Up	2049
<u>X02812</u>	TGFB	transforming growth factor, beta 1	Up	1535
<u>U21092</u>	TRAF3	TNF receptor-associated factor 3	Up	1407
<u>U05875</u>	IFNGR2	interferon gamma receptor 2	Up	1372
<u>X67325</u>	IFI27	interferon, alpha-inducible protein 27	Up	1182
<u>J03909</u>		gamma-interferon-inducible protein; IP-30	Up	1076

**Table 2: Down-regulated genes in HMTV env+ cells**

Acc #	Gene symbol	Protein/gene	Ratio	Diff
<u>U02687</u>	FLT3	fms-related tyrosine kinase 3	-6.20	-2143
<u>X74295</u>	IGA7B	integrin alpha 7B	-5.13	-2249
<u>X53587</u>	ITGB4	integrin beta 4	-4.80	-2384
<u>M34671</u>	CD59	CD59 molecule, complement regulatory protein	-3.60	-2551
<u>L25081</u>	RHOC	ras homolog gene family, member C	-3.59	-2341
<u>U89278</u>	PHC2	polyhomeotic-like 2	-3.53	-1250
<u>X16277</u>	ODCI	ornithine decarboxylase I	-2.87	-1295
<u>AF029670</u>	RAD51C	RAD51 homolog C	-2.61	-1713
<u>M20430</u>	HLA-DRB1	MHC class II HLA-DR-beta	Down	-4288
<u>J04111</u>	JUN	c-jun proto-oncogene; transcription factor AP-1	Down	-1745
<u>U70310</u>	FANCG	DNA repair protein XRCC9	Down	-1514
<u>M59911</u>	ITGA3	integrin alpha 3	Down	-1393
<u>M97934</u>	STAT2	signal transducer and activator of transcription 2	Down	-1248
<u>L38518</u>	SHH	sonic hedgehog	Down	-1183
<u>X51521</u>	VIL2	ezrin; villin 2	Down	-1177
<u>L07515</u>	CBX5	chromobox homolog 5; heterochromatin protein homolog 1 (HPI)	Down	-1153
<u>M15400</u>	RBI	retinoblastoma 1	Down	-1109
<u>M54995</u>	PPBP	pro-platelet basic protein	Down	-1100
<u>X54199</u>	GART	trifunctional purine biosynthetic protein adenosine 3	Down	-1092
<u>U47686</u>	STAT5 A +B	signal transducer and activator of transcription 5 A+B	Down	-1072

## Methods

MCF-7 cells were obtained from American Type Culture Collection (ATCC) and were propagated in vitro as recommended by the provider and as described in previous publications (1, 5). To determine whether the viral protein was expressed in our MCF-7 cells, western blotting was used. Protein lysates were prepared from approximately  $1 \times 10^7$  cells. Equal amounts of protein from each sample were loaded onto an SDS-PAGE-10% polyacrylamide gel, followed by transfer to PVDF membranes. Western blot analysis was performed using mAbP2 (a monoclonal antibody against a peptide of the Env protein), and mAb-tubulin as primary antibodies (Sigma Aldrich). Proteins were visualized using horseradish peroxidase-labeled sheep anti-mouse IgG (GE Healthcare Bio-Sciences Corp.) as a secondary antibody followed by enhanced chemiluminescence (GE Healthcare Bio-Sciences Corp.).

The expression profile was studied using the Atlas Human Cancer 1.2 cDNA expression array; a nylon membrane printed with 200–600 bp long fragments of 1176 characterized genes involved in cancer, 9 housekeeping genes and 6 negative controls (Clontech, CA). These conditions were described in detail in a previous publication [25]. Briefly, RNA was extracted and labeled with Atlas pure total RNA labeling system and hybridized to an Atlas Human Cancer 1.2 cDNA expression array (Clontech, CA) according to the manufacturer's instructions. Both cell sublines were probed twice in separate assays, and the accuracy of each duplicate was assessed by Pearson's cor-

relation coefficient based on the adjusted intensity of all genes spotted on the membrane.

Hybridizations with 30 µg of total RNA were performed according to the manufacturer instructions. The hybridized membranes were exposed onto a phosphorimager screen and were read using a phosphorimager reader (Molecular Dynamics). The scanned images were aligned and analyzed using AtlasImage 2.01 software (Clontech). When averaging or comparing samples, the adjusted intensity signal was normalized using the global normalization mode featured in the software. We reported only those genes whose ratios of differential expression were 2.5-fold or more, or genes that were undefined for one type of sample, but were detected on the other. (Undefined genes are those whose intensity were below the signal threshold) In the later event, when we lack a numerical value for the ratio, it was defined as being "up" or "down". Furthermore, for each gene we stated the difference (diff) in adjusted normalized intensity between the two cell lines.

Accession number (Acc#), gene symbol and protein or gene name are according to GeneBank.

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